

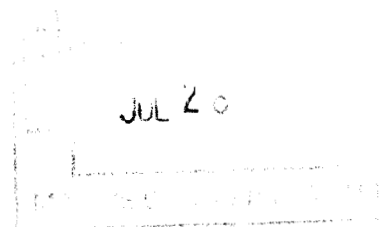
**ENTEROCOCCI BACTERIA: EFFECTS OF TIDES, RAINFALL, AND TIME OF WEEK
ON LEVELS OF A NEARSHORE POLLUTION INDICATOR
WAI OPAE TIDEPOOLS, HAWAII**

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ABSTRACT: *Enterococci* bacteria (EC) are the standard pollution indicator for near shore marine recreational waters in Hawaii. These bacteria originate from human and animal feces as well as tropical soils and they are known to cause gastroenteritis in swimmers. In a preliminary study on the east side of the Big Island at Wai Opae Tidepools, EC concentrations exceeded acceptable safe levels as defined by both the Hawaii State Department of Health and the Environmental Protection Agency. Preliminary sampling from the spring of 2003 showed high and low levels of bacteria within the same tidepools at different times. Two hypotheses resulted from these early observations: Quantities of EC are higher during weekends than on weekdays; Quantities of EC are higher at low tide than at high tide. Membrane filtration techniques were used to quantify EC colonies in 108 samples collected from August 2003 to February 2004. Data was analyzed in three different time periods: August; August - February; and November - February. To isolate the source of the bacteria, water was also collected and filtered for suspended particulate matter (SPM) concentration and particulate organic matter (POM) percentages. A negative correlation between SPM and EC indicated that soil was not a source of the bacteria. Several tidepools consistently exceeded state and federal standards. Statistical analysis showed one significant difference between EC levels on weekdays and weekends in the winter months and no significant difference between high tides and low tides. Rainfall data from 48 hours prior to sampling was positively and significantly correlated with EC and therefore it is an important predictor of EC in seawater. The majority of the houses in the community adjacent to the tidepools are equipped with cesspools and percolation of sewage effluent from them is thought to be the primary source of the EC the tidepools.

Introduction

Coral reef ecosystems are of ecological, biological, physical, and economical importance worldwide (Moberg and Folke 1999). In addition to natural disturbances such as hurricanes and high surf they are subjected to increasing anthropogenic impacts such as pollution via run-off, sewage inputs, eutrophication, overfishing, coastal development, sedimentation, and global warming. According to Wilkenson (2000) it is estimated that a combination of physical, chemical, and biological stresses will cause the decline of between 40% to 60% of the world's coral reefs over the next fifty years unless appropriate steps are taken (Lesser 2004).

Eutrophication in the marine environment is a global focus and currently the topic of many scientific studies. An experiment at the Great Barrier Reef showed responses to nutrient enrichment in corals. These included coral mortality, stunted growth, and reductions in skeletal density, settlement of coral larvae, and numbers of successfully developed embryos (Koop et al 2001). In the Bahamas, the effects of nutrient enrichment and the importance of the links between grazers (top down control) and nutrient caused macro-algal blooms (bottom up control) was investigated and overall, nutrient enrichment is a more important factor in controlling algal biomass than herbivory (Lapointe et al 2004). Sediment core samples from the Gulf of Mexico provided evidence that coastal eutrophication may also be directly linked to harmful algal blooms (Parsons and Dortch 2002). Finally, marine eutrophic systems can be characterized by a higher degree of viral infection; increasing numbers of viruses usually occur with increasing numbers of bacteria (Danovaro 2003).

Common sources of excess nutrients are fertilizers and sewage. Sewage not only contributes nutrients to an area but it can also introduce pathogens. It has been insinuated that diseases such as white pox in corals are linked to sewage inputs in the Florida Keys (Lesser 2004). Sewage can also affect the abundance, mortality, fecundity, and size of fishes as well as lead to toxic effects, increase susceptibilities to infections, and alter behavioral responses in fishes (Gray 1989; Adams et al 1993; EPA 1993a in Smith 1999). The eutrophication and reef community structure shifts in Kaneohe Bay reefs on Oahu have been linked to sewage input and then diversion within the past several decades (Hunter 1995).

Currently, the Environmental Protection Agency (EPA) and the Hawaii State Department of Health (HDOH) use the enteric bacteria *enterococci* (EC) as an indicator of near shore pollution of marine recreational waters (State of Hawaii 2003). Although not all are pathogenic, EC bacteria are found abundantly in wastes with human contributions where pathogenic organisms, such as viruses are common (Noble 2003). EC can inhabit the intestinal tract of humans and animals such as chickens, and dogs. Tropical soil is also a natural substrate for the growth of EC (Hardina and Fujioka 1991). EC is a genus of bacteria previously grouped with fecal streptococci (Pinto 1999) containing at least 28 different species. Two of these species can cause complicated abdominal infections (gastroenteritis in swimmers), skin and skin structure infections, urinary tract infections, and infections in the blood stream. They are tolerant of high salt concentrations, as well as low levels of detergents and these two species have developed resistance to antibiotics which makes them potentially life-threatening (Hancock 2000).

The Hawaiian Islands are home to the majority of the coral reefs in the United States with Oahu and the Big Island of Hawaii boasting the highest coral biodiversity in main Hawaiian archipelago (Gulko et al 2000). Several Marine Life Conservation Districts (MLCD) have been established in Hawaii but only one, recently declared (June 2003), exists on the east side of the big island. Wai Opae Tidepools (Kapoho) are a "No Take" MLCD located in the Puna district just south of Cape Kumukahi (Fig.1). They are a reef flat exposed to the Northeast trade winds coming off the ocean and are bordered on land by a community called Vacationland.

Wai Opae Tidepools have been used frequently by the University of Hawaii to conduct a variety of research experiments. Past unpublished studies have shown these tidepools to be abundant and diverse in corals and other marine organisms. The West Hawaii Aquarium Project also has unpublished data indicating the tidepools are utilized as a fish nursery for tropical reef fishes. Of their 26 sample locations island wide, these tidepools showed the highest abundances of juvenile fish. The area is highly accessible to human use therefore; water quality is an important issue for both humans enjoying Wai Opae Tidepools and marine organisms residing there.

Prior to this experiment, the Vacationland Hawaii Community Association (VHCA) sponsored water quality tests at Wai Opae for coliform and EC on May 27, 2002 and EC on February 26, 2003. Results from both dates showed levels exceeding HDOH and EPA standards in some inland and coastal tidepools. Jill Ley (2001) also cited findings of high bacterial levels at Kapoho in studies by Thomas (1989) and Miller (1991). EC colony counts should not exceed a geometric mean of more than 7 colonies

per 100ml of seawater within 300 meters of the shoreline, with the stipulation that at least 5 samples are taken within a thirty day period (State of Hawaii, 2003).

In addition to Wai Opae, Hilo Bay (~25 miles north) and Kapoho Bay (~1 mile north) have also been water quality study sites. Both Hilo and Kapoho study results showed high levels of EC and fecal coliforms. It was suggested that freshwater run-off and /or percolation from commercial and residential cesspools were the primary sources of sewage (bacteria) input into Hilo Bay (Dudley and Hallacher 1991). A Kapoho Bay study by the HDOH in 1984 confirmed rapid cesspool seepage into tidepools by dye testing, and subsequent high levels of fecal coliforms at tidepools within the coastal community and nearshore of the community. In 1973, John Ford concluded that at Kapoho bay the cesspool seepage was occurring through fractures in the regolith, lava tubes and from freshwater stream discharge. A combination of the porous nature of the lava rock and the cesspools dug into the rock under coastal dwellings allows considerable seepage of untreated sewage into tidepools. Similar to Kapoho Bay residences, most homes in Vacationland have lava rock lined cesspools. Only a few homes have septic systems, with only one having a triple stage treating process. Therefore it is possible that sewage seepage into the MLCD is occurring from cesspools on a daily basis.

The preliminary results from tests at Wai Opae Tidepools showed a need for further sampling. Previous sampling was conducted sporadically and did not follow the protocol required by the HDOH. Based on previous results, ten tidepools were chosen for further sampling. Five were located inside the MLCD and the other five were outside in an area designated OPEN (Fig. 1). They were sampled with the intention of showing

that EC levels are higher on weekends than on weekdays and that EC levels are higher at low tides than at high tides. The first hypothesis assumes that more vacation rentals are occupied on the weekends and that sewage has a short residence time in the cesspools. The first set of tests also adhered to the HDOH protocol by producing a geometric mean of EC based on five nonconsecutive sample days within a thirty day period. The second hypothesis is important in the determination of the safest times for swimming in the tidepools as well as the degree of impact flushing rates and residence times of sewage have on the area.

Methods

Colonies of EC in seawater samples at Wai Opae Tidepools were quantified using membrane filtration techniques from the *Standard Methods for the Examination of Water and Wastewater, 19th Edition* (1995) and the HDOH with methods from the *US EPA Microbiological Methods for Monitoring the Environment, Water, and Waste* (1978). Refer to the specific Water Quality Methods in appendix I for details.

Hypothesis I

To address the weekend versus weekday hypothesis, ten, 500ml samples were taken from ten previously selected tidepools (Fig. 1) on six days during a thirty day sampling period, spanning the month of August 2003. Sampling occurred on three non-consecutive weekend days and three non-consecutive weekdays at low tide, in accordance to the HDOH water quality standards for sampling (State of Hawaii, 2003). Within three to six hours of sample collection, samples were filtered and filters were placed on bacteriological growth media and incubated. After the initial growth period colonies were counted and then divided among various media and incubated for

verification. Each sample required appropriate tides and five to six days of analysis in the lab, therefore scheduling included all days of the week, Monday-Sunday. Salinity and temperature measurements were taken using the YSI in situ. Daily rainfall data was recorded in Vacationland at 07:00 by Ellen Corbet for the National Weather Service.

Hypothesis II

To determine if a significant difference existed between high tides and low tides, samples were collected at different time intervals than described above, however the lab analysis remained the same. Only tidepools (3,8,14,15) (Fig. 1) with high levels of bacteria from hypothesis I results were used in the testing of hypothesis II (Table 2). These tidepools were sampled twice in one day, once at the highest tide and once at the lowest tide occurring during daylight hours. They were sampled on six different days from November 2003 to February 2004.

In addition to the collection of water for bacteriological purposes for both hypotheses, 1000ml of water was collected and filtered in the lab for analysis of suspended particulate matter (SPM) and particulate organic matter (POM) (Parsons et al. 1984). Whatman 47mm 934-AH (Fisher cat#1827 047) glass fiber filters were pre-dried and pre-massed in aluminum tins. After filtration, filters were replaced to the pre-massed aluminum tins and dried for 24 hours at 60° C and massed. They were returned to the oven in the tins and burned at 500° C for another 24 hours and massed again. SPM and POM were then determined using the following equations:

$$\text{SPM (mg/l)} = (\text{pre-massed filters}) - (\text{post } 60^{\circ} \text{ C drying mass})$$

$$\text{POM (\%)} = \frac{(\text{post } 60^{\circ} \text{ C mass w/filter}) - (\text{post } 500^{\circ} \text{ C mass w/filter})}{(\text{SPM})} \times 100$$

Statistical Analysis

ANOVAs were used to examine the differences between the factor time of week and the response EC concentration. For the first hypothesis three date ranges were analyzed with ANOVAs, August 2003, August 2003-February 2004, and November 2003-February 2004. These ranges were chosen to compare samples within seasons and overall. ANOVAs were also used to determine differences between the factor, tide, and response, EC concentration, for November-February data. Regression analyses were used to determine significance of rainfall, salinity, temperature, SPM, POM in predicting EC concentration. Regressions were used to compare EC concentrations versus rainfall over 24 hours, 48 hours, 72 hours, and 7 days.

Results

Three sets of data were used to address null hypothesis #1: Quantities of EC per 100ml sea water are equal on weekends and weekdays. The first set was collected during August 2003. Ten tidepools were sampled each day on 6 nonconsecutive days during a 30 day period at low tide, yielding a total of 60 samples. No significant differences were found using an ANOVA (Table 1). Additional data collected to address the second hypothesis was also used for the first with a combined sample size of 108 and again no significant differences were found using an ANOVA. However, the third set of data from November to February from tidepools (TP) 3, 14, and 15 combined (N=36) showed a significant difference between weekend and weekday EC (Table 1; Fig. 2). Because of inconsistencies in colony counts in TP #8, it was removed from this data set for analyses.

Using an ANOVA, data from TP 3, 14, and 15 in November, January, and February (N=48) were analyzed for the second null hypothesis: Quantities of EC per 100ml sea water are equal at high tide and low tide. No significant difference was noted and the null hypothesis was accepted (Table 1).

In the pooled data set (N=108) EC counts ranged from 0 to 262 colonies 100ml⁻¹ SW, although the high counts were usually from 100-130 which are approximately four times the exceeded EPA standards (35cfu100ml⁻¹ SW) and sixteen times the HDOH standards (7cfu100ml⁻¹ SW). According to the HDOH standards seven of ten tidepools located near shore are not safe for recreational swimming (Table 2).

The regression analysis of 48 hour rainfall versus EC concentration in the pooled data set yielded the highest R squared value (51.5%) and the lowest P value (0.008) (Fig. 3). Correlations between rainfall and EC are significant after 24 and 48 hours (Fig. 7; Fig. 3), but cease being significant after 7 days. Over a 48 hour period, the highest rainfall occurred in January (2.33in³) and the lowest in August (0.06in³).

A regression of salinity versus EC shows a negative linear correlation; as salinity decreases in seawater, colony count increases (Fig. 4). November to February data showed a significant difference in salinity between high tide and low tide (Fig. 6). Salinities ranged from 11.2‰ in TP 15 at low tide to 34.5‰ in TP 3 at high tide.

SPM (mg/l) values ranged from 9.7(mg/l) in TP 15 to 51.5(mg/l) in TP 8. The percent of particulate organic matter within the SPM samples ranged from 21.2% in TP 3 to 53.0% in TP 15, with a mean of 37.2% (st.dev.=5.93) in all tidepools. Although the R-squared value (26.8%) is small (Fig. 5), SPM does have a significant negative

correlation with EC per 100ml sea water ($P=0.000$). As the SPM increases the EC concentration decreases.

Discussion

Many of the near shore tidepools at Wai Opae consistently exceeded EC EPA standards ($35\text{cfu}100\text{ml}^{-1}$ SW) and HDOH standards ($7\text{cfu}100\text{ml}^{-1}$ SW) from August to February. Other studies have reported failures in terms of $>104\text{cfu}100\text{ml}^{-1}$ SW (Noble, 2003; Schiff, 2003) in California where samples ranged from approximately 200-6,000 $\text{cfu}100\text{ml}^{-1}$ SW. These levels found along Santa Monica Bay beaches and present a worst case scenario. Locally, the Hilo Bay study reported 8 out of ten sites had EC concentrations continuously exceeding state standards (Dudley and Hallacher 1991). Cesspool leaching is most likely the major contributor of EC to the tidepools. During high tide flooding and excessive rainfall, fecal matter from dogs, chickens, and humans (no facilities available facilities) may contribute EC via run-off. Although EC grows naturally in tropical soils the inverse relationship between EC and SPM (Fig. 5) shows that sediment is probably not a major source of EC. In order to confirm cesspool leaching dye tracer studies are imperative.

SPM samples show that on the average there is 25.7 mg/l (st.dev.=7.73), 37.2% (st.dev.=5.93) of which is particulate organic matter (POM). According to a study completed in the Bohai Sea, high levels of SPM are approximately 50mg/l (Jiang et al 2004), therefore Wai Opae samples are relatively lower. Land bordering Wai Opae tidepools is composed of mostly lava rock and small amounts of sediment from erosion of lava rock; therefore tidepool sediment loading with high EC concentrations as a result of run-off is unlikely.

It is possible that during periods of low rainfall, light wind, and small surf, a low tide will yield the highest levels of bacteria. Initially, after eight samples in a low rainfall period during November (2003), there were significantly higher levels of EC present at low tide versus high tide ($P < 0.05$). However, upon completion of 16 additional tests in higher rainfall periods during January and February (2004), there was no significant difference shown between high tide and low tide, indicating that rainfall became a dominant factor over tidal cycle when rainfall was high (Table 1). Observations of a freshwater lens in the tidepools are common on sunny and calm days. A salinity versus EC regression from shows that salinity is also an important predictor of EC (Fig. 4). Colony count is higher when a freshwater lens exists. November to February samples also show that salinity is significantly different between high tides and low tides (Fig. 6).

Wet and stormy weather with increased rainfall has been an important factor in other similar bacteriological studies. A study in Southern California compared indicator bacteria failures from dry winter and summer data and stormy data. EC failed the single sample standards most often in all weather conditions with the highest failure percentage during storm events (Noble et al 2003). In the 1991 Hilo Bay study, a positive correlation between rainfall and EC occurred often at most sites (Dudley and Hallacher 1991). A Santa Monica study also compared wet and dry conditions with indicator bacteria failures and most EC failures were documented during wet weather (Schiff 2003). However, they also targeted storm drains (run-off) as an untreated contaminant source. If run-off was a major contributor of EC at Wai Opae, a shorter delay between rainfall and the subsequent increased EC would be expected. While the 24 hour delay was significant (Fig. 7) and indicated that run-off was important, the 48

hour delay was the most significant (Fig. 3) and indicated some kind of percolation, probably from cesspools, was a more influential factor. Additionally, increased light intensity has a degrading effect on EC (Alkan 1995), and it is possible that rainy conditions may inhibit photo-degradation due to cloud cover above, and turbidity under water during and after seepage of cesspools.

Periods of high wind and surf may also dilute EC and increase the flushing rate of the tidepools. The prevailing 15-20 knot tradewinds at Wai Opae maintain the 3-5 ft choppy surf outside the reef crest for the majority of the year and these could be responsible for a regular flushing effect on the tidepools. Hydrology experiments would be helpful to further investigate fluxes in the tidepools. During the winter or off-season months, along with increased rainfall, a weekend effect is also observed. More frequent weekend (vs weekday) vacation renters may be responsible for the difference between weekdays and weekends. During the popular vacation time of summer, rentals may be occupied at any time during the week. Rentals may be less occupied during the winter months because school is in session and the rainy weather may deter weekday users however, data on rental occupation have not been collected to date. Another possibility is that the pulse of residential EC input is seen more clearly during winter months with less overall visitors and more precipitation (Table 2).

These near shore sites are frequently used by residents and visitors, and according to the state, the tidepools would be deemed unsafe for near-shore recreational use. This study raises safety concerns because swimmers exposed to high levels of EC in some tidepools could contract gastroenteritis (Cabelli et al 1982). In order to infect, EC must be allowed to colonize mucosal surfaces. Once infection

occurs, EC has multiple antibiotic resistances and some strains show increasing resistance with time. This leads to problems involved in treating the infection (Fischetti et al 2000). When EC are present in the tidepools, it is possible they are accompanied by viruses and other pathogens found in untreated wastewater. The long term effects of excess nutrients from sewage effluent are marine eutrophication and mucilage formation, which is often related to the development of large viral assemblages (Danovero 2003). Swimmers should be most weary of tidepools 14 and 15 (Table 2; Fig. 1) because they consistently boast the highest levels of EC.

At Wai Opae, unpublished studies have not revealed that the tidepools are presently in danger of eutrophication. Unfortunately, no long term studies necessary to show these changes in the environment have been conducted at the tidepools. To date, studies done show few direct links exist between EC infection and marine and freshwater organisms. A giant freshwater prawn *Macrobrachium rosenbergii* has increased susceptibility to EC infection as dissolved oxygen levels decrease (Cheng 2002). Anti-microbial activity exists in some marine species of Molluscan egg masses against EC, *E.coli*, and *Staphylococcus aureus* (Benken 2001), but long-term effects are unknown.

If sewage effluent is percolating into Wai Opae Tidepools from cesspools and being washed in via run-off, long term effects could lead to signs of eutrophication and disease. Coral reef waters are known to be oligotrophic and compared to nutrient limited algae, corals are slow growing. Nutrient enrichment not only gives a competitive edge to algae versus coral growth but it also can increase phytoplankton biomass (Parsons and Dortch 2002) resulting in decreased light intensity and increased biological oxygen

demand which may also affect corals and the stability of the ecosystem (Guzman et al in Richmond 1993). For decades Kaneohe Bay on Oahu has been a study site used to document the impacts of pollution in Hawaiian waters. Over the past 60 years it has been subjected to stress resulting from increasing agriculture, urbanization, and sewage outfalls (which are currently diverted). Although Wai Opae and Kaneohe Bay greatly differ in levels of impact both are located on the windward sides of Hawaiian Islands. The studies in Kaneohe Bay did result in the identification of some potential indicators of eutrophication such as increased nutrients levels, turbidity, and phytoplankton abundance, as well as a community structure dominated by *Dichtospheria cavernosa* (bubble alga) and filter or deposit feeders (Hunter 1995). Although bubble algae may not endanger Wai Opae, there are other invasive algae in Hawaii, such as *Hypnea musiformis* (Gulko et al 2000), that may be of concern. These factors will be important study foci in the future of Wai Opae Tidepools.

Future studies at Wai Opae should include marine monitoring for indicator species, dye tracer testing to obtain confirmation of leaching and sewage residence times, and hydrology experiments. Currently University of Hawaii graduate students, Kaeo Duarte and Ryan Okano, are beginning hydrology experiments funded by the EPA at Wai Opae. For bacteriological analysis, changes in experimental design may also yield more precise results in showing significant differences in EC levels between tides. More frequent sampling of only two tidepools (14,15) with consistently high levels of EC and low salinities would give less variation about the means. Some tidepools (1,2,5,8,9,12,13) have consistently lower levels of EC and should therefore be omitted from this part of the experiment. With an increase in visitors and residents, the

Vacationland community at Wai Opae and the HDOH will eventually need to seriously address changing cesspools into septic tanks or diverting sewage away from the ocean to prevent disease and infection in humans and marine life as well as eutrophication. Robert Richmond reminds us, "A general rule for islands: Whatever is used on land today ends up in the aquifer or coastal zone tomorrow" (1993).

Acknowledgments

VHCA: Linda and Kirk Flanders, Karen Klein, Claudia Charlos, Ellen Corbet.

UHH: Dr. Michael Parsons, Dr. Leon Hallacher, Dr. Marta DeMaintenon, Dr. Walter Dudley, Dr. Roger S. Fujioka, Dr. Paul Haberstroh, Debbie Scott, John Coney, Randy Schneider, Tania DeCambra. HDOH: Ronald Kaya, Lori Ueno, Rae Harada, Randee Tubal.

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TABLE 1. ANOVA results for comparisons of quantities of *enterococci* colonies per 100ml⁻¹ seawater. No significant differences between colony count at high tides and low tides. One significant difference was found between colony count on weekends and weekdays. Significant differences (P<0.05) denoted by *. Bold print shows average rainfall during that period. Factors: High Tide=HT; Low Tide=LT; Weekend=WE; Weekday=WD.

FACTOR		SAMPLE SIZE	MEANS	P Value
November				
January	HT	N=18	26.28	0.177
February				
03-04	LT	N=18	45.28	
0.79				
August	WE	N=30	22.73	0.765
03				
0.37	WD	N=30	26.10	
August-				
February	WE	N=54	32.54	0.143
03-04				
0.58	WD	N=54	20.76	
November				
January	WE	N=18	52.89	0.012*
February				
03-04	WD	N=18	18.67	
0.79				

TABLE 2. August 2003 enterococci (100ml^{-1} SW) geometric mean results from five nonconsecutive samples of ten different tidepools within thirty days. Tidepool 3 consistently lower with the exception of one extremely high count. Means exceeding: EPA standards ($35\text{col}/100\text{mlSW}$)-bold print; Hawaii State Department of Health Standards ($7\text{col}/100\text{mlSW}$)-underlined.

Tidepool	Mean enterococci colonies (100ml^{-1} SW)
1	<u>19</u>
2	<u>10</u>
3	61
5	6
8	<u>20</u>
9	<u>18</u>
12	2
13	0
14	42
15	63

Fig. 1. Map of Sample Locations at Wai Opae Tidepools on East Side of the Big Island of Hawaii.

Fig. 2. Significant difference shown between Weekend and Weekday samples from November, January, and February (2003-2004).

P value = 0.012

Fig. 3. Regression analysis of rainfall (") from 48 hours prior to sampling as a predictor of *Enterococci* colonies. P value= 0.005

Fig. 4. Regression analysis of Salinity (ppt) as a predictor of *Enterococci* colonies (100ml⁻¹ sea water). P value = 0.000

Fig. 5. Regression analysis of Suspended Particulate Matter (mg/l) as a predictor of *Enterococci* colonies (100ml⁻¹ sea water). P value = 0.000

Fig. 6. ANOVA graph of significant difference in High Tide and Low Tide salinities. P value = 0.000

Fig. 7. Regression analysis of rainfall (") from 24 hours prior to sampling as a predictor of *Enterococci* colonies. P value= 0.001

Fig. 1



Photo by John Coney

Fig. 2

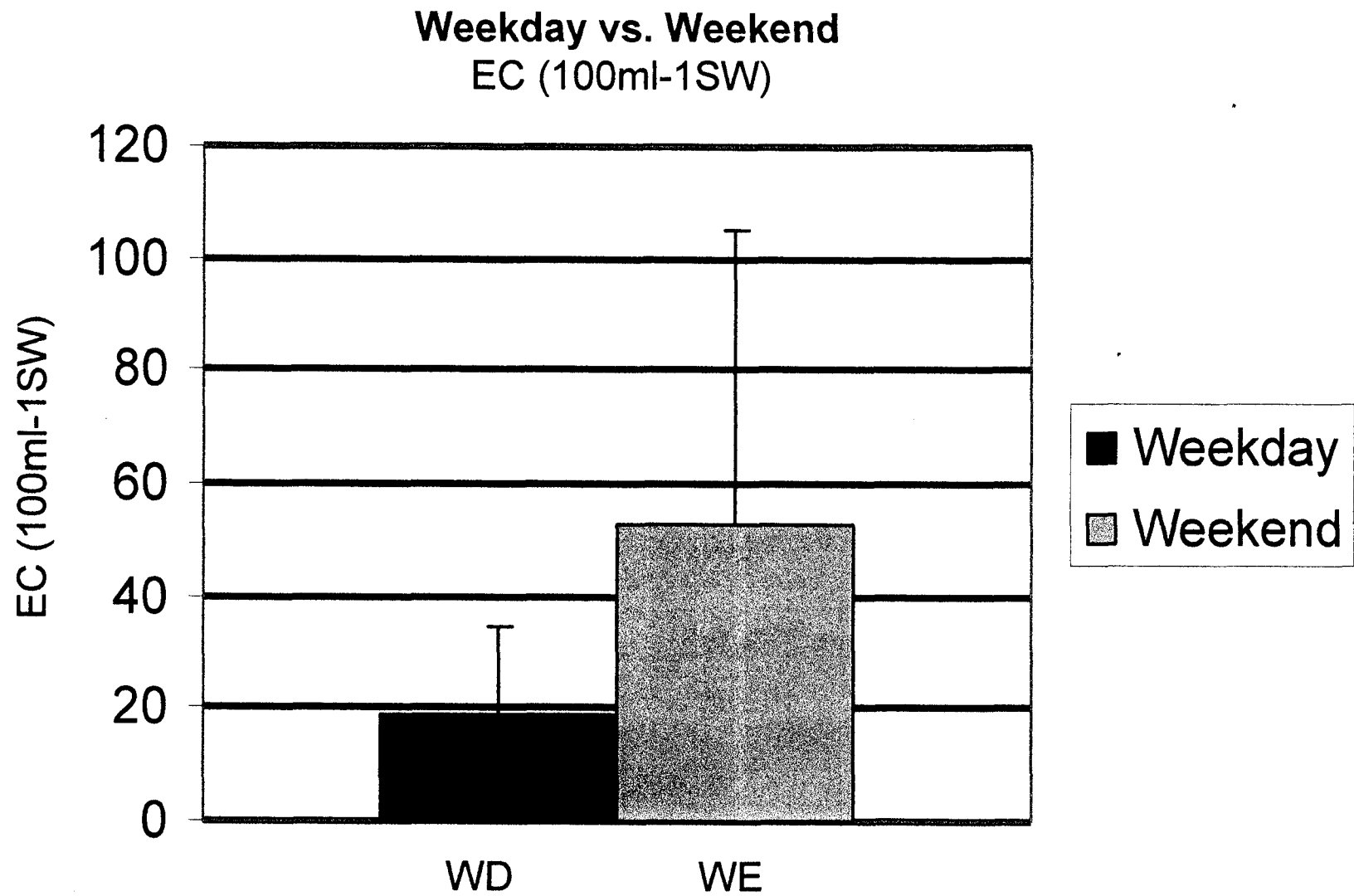


Fig. 3

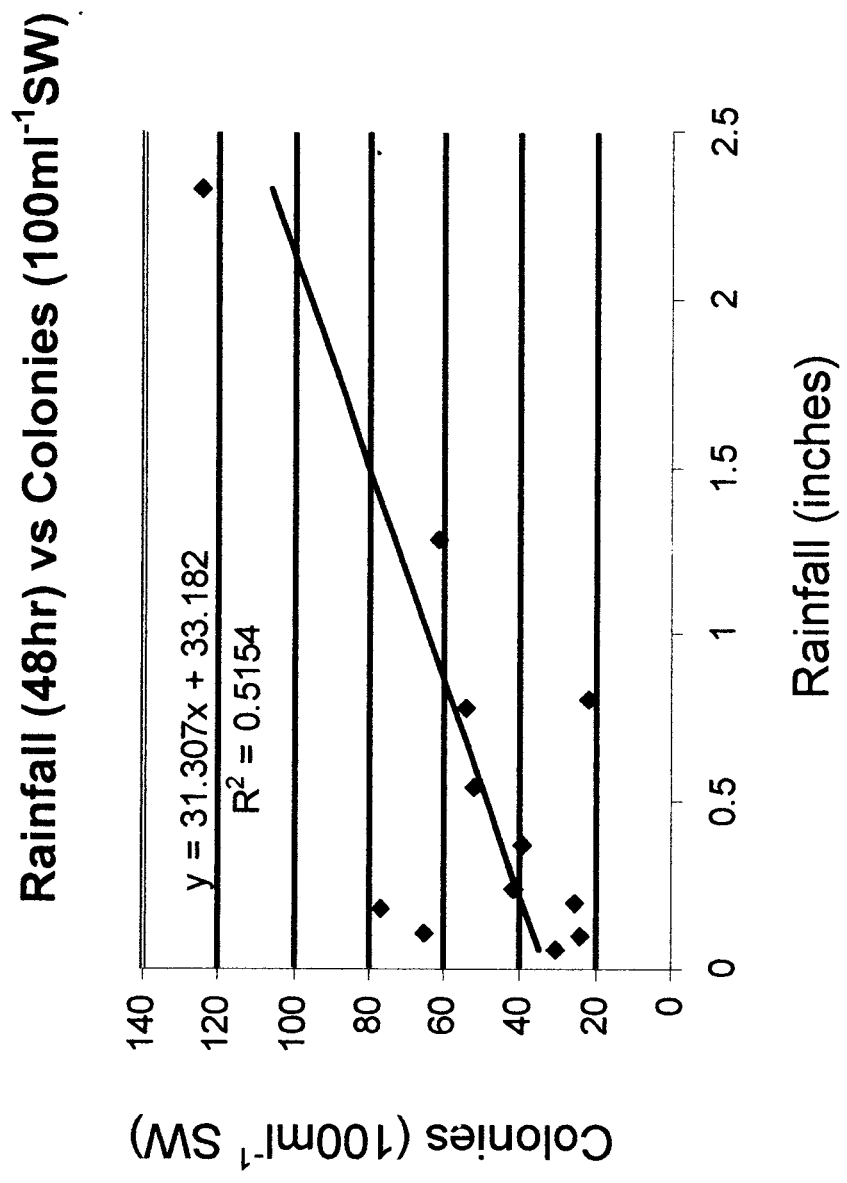


Fig. 4

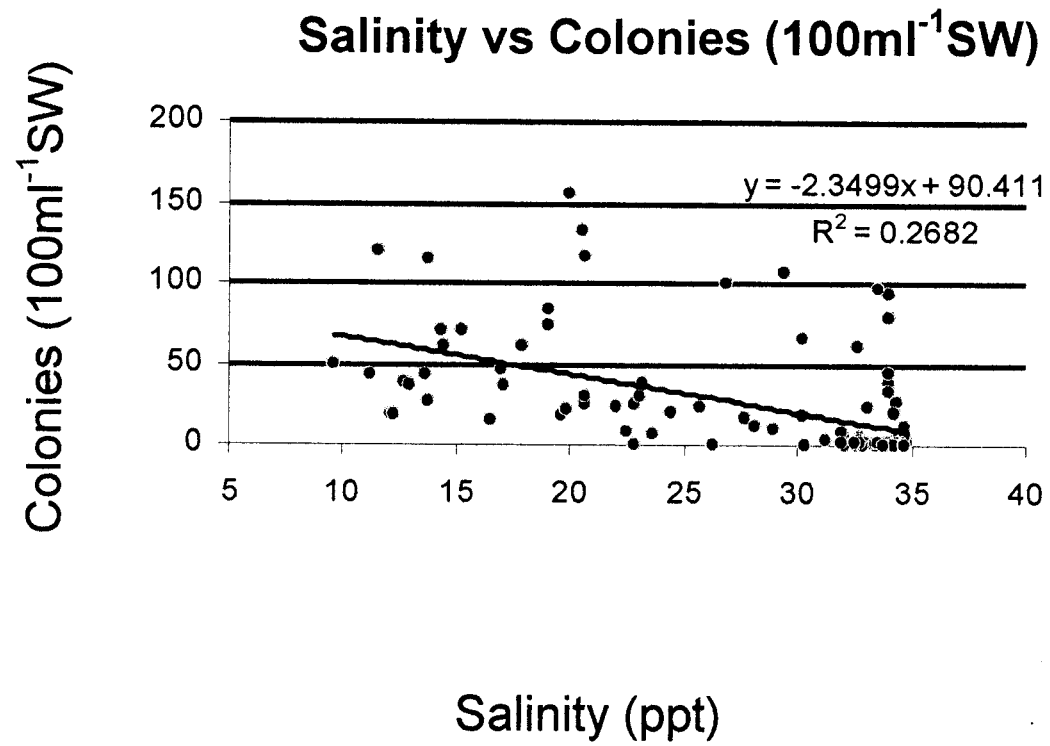


Fig. 5

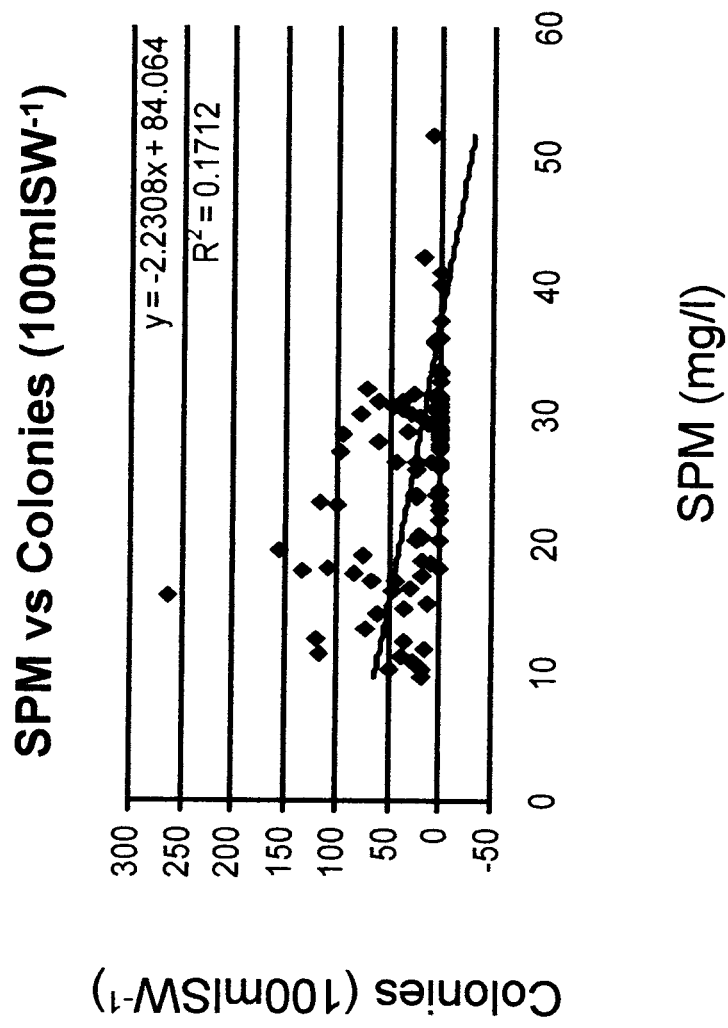


Fig. 6

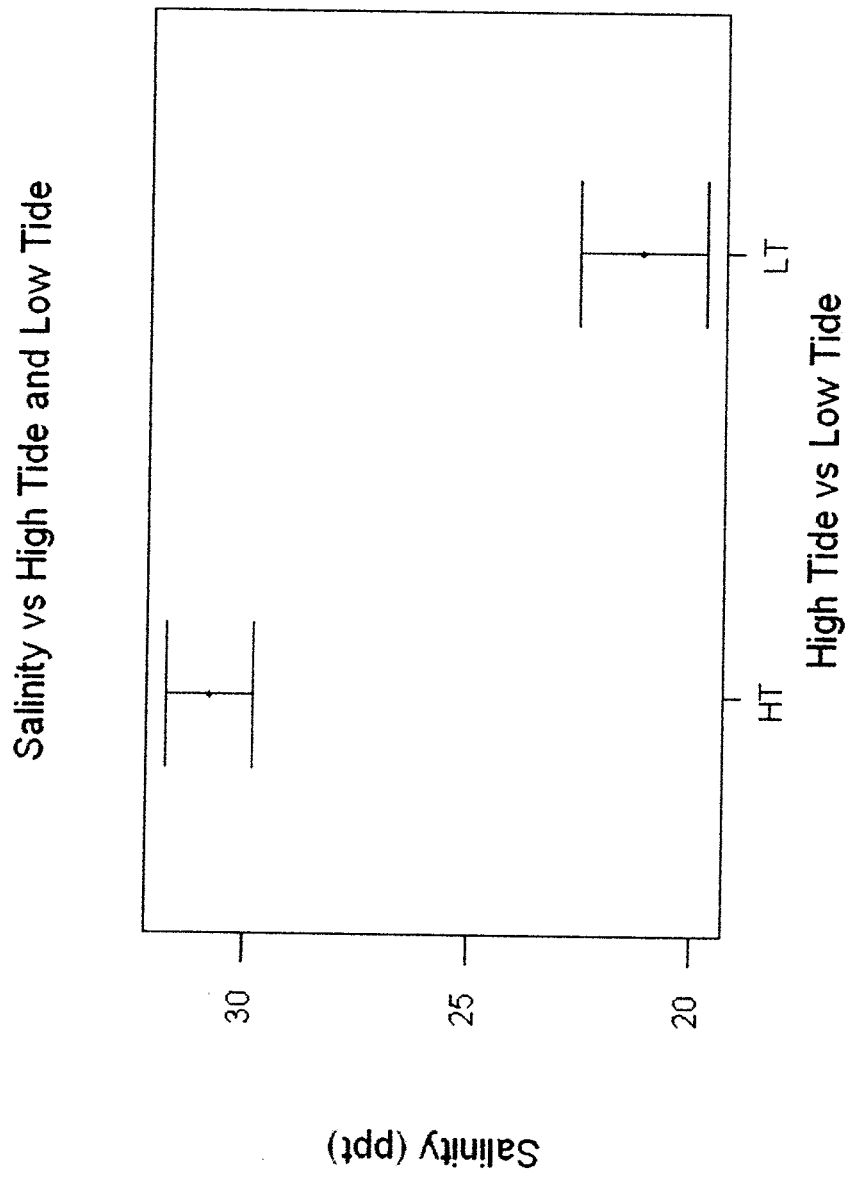


Fig. 7

